Hypothesis

Oxidation conspires with glycation to generate noxious advanced glycation end products in renal failure

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Abstract

The causes of long-term complications of uraemia are yet to be fully elucidated. It has recently been demonstrated that renal failure is associated with a dramatic elevation of advanced glycation end products (AGEs). These products are the result of the non-enzymatic Maillard reaction linking a protein amino group with a glucose-derived aldehyde group. A mild rise of AGEs is associated with normal ageing. Proteins with a slow turnover such as matrix proteins are thus modified and removed by an AGE-specific receptor-mediated process thought to be a part of normal tissue remodelling. A more striking rise of AGEs is observed in diabetic patients as a result of sustained hyperglycaemia. A greater variety of proteins is thus modified leading to tissue damage through alteration of tissue protein structure and function, stimulation of several cellular responses, or generation of reactive oxygen species. In uraemia, the rise of AGEs is even more marked than in diabetics and is associated with a variety of tissue disorders including vascular damage, dyslipidaemia, and β₂-microglobulin amyloidosis. AGE accumulation in uraemia does not result from hyperglycaemia. Identification of its cause as well as of the involved precursors should contribute to the understanding of uraemic toxicity and open new therapeutic approaches. In this presentation, we propose the hypothesis that AGE generation is enhanced by an increased oxidative stress associated with uraemia. Under these conditions, a variety of compounds, both related and unrelated to glucose, may contribute to the advanced glyoxidation of proteins. In uraemia, AGEs could be taken as a marker of oxidative stress damage to proteins.

Key words: advanced glycation end products; uraemic complication; redox; glycoxidative stress; pentosidine

Renal failure is associated with the retention of a variety of toxic compounds responsible for the uraemic syndrome. Initially, most of the symptoms of acute uraemia were attributed to high blood urea levels and to acid–base and electrolyte disorders. Subsequently, when it became clear that urea was not very toxic, a variety of other protein-derived catabolic products were incriminated. Among them, only a small minority has been convincingly associated with certain symptoms of acute renal failure. The development of renal replacement therapy, and especially dialysis, has led to identification of a series of complications associated with long-term chronic renal failure (indeed the clearance capacity of three weekly dialysis is equivalent to a creatinine clearance of approximately 10 ml/min). These complications include a depressed immune response to infection, cardiovascular disorders including a specific uraemic cardiomyopathy, and dialysis-related amyloidosis. Their specific causes are currently being investigated. A number of compounds have been incriminated, e.g. parathyroid hormone, β₂-microglobulin (β₂m), and p-cresol. In many instances, however, the causal role of these ‘toxins’ remains debated.

The discovery that, in humans, long-lived proteins undergo progressive rearrangements has opened new avenues. These products, called advanced glycation end products (AGEs), result from the Maillard reaction which, over a period of several months, links proteins with glucose-derived aldehydes resulting in Schiff bases, which are subsequently transformed through molecular rearrangements into Amadori products and eventually into irreversible AGEs [1]. This process is so slow that it affects only proteins with a slow turnover such as matrix proteins. Once modified, these proteins lose some of their physiological functions and have to be removed. Indeed, AGEs are recognized by specific receptors [2] found in cells such as macrophages [3] and, as a result, are broken down, allowing appropriate tissue remodelling. It is not surprising to find that AGE levels slowly increase with age in a variety of collagenous structures, including the skin and lens as well as in the serum [4].
In 1988, the group of Vlassara demonstrated a marked increase of AGEs in the tissues of diabetic patients [1]. This increase was ascribed to hyperglycaemia: indeed a correlation was found between the levels of fructoselysine, a direct result of hyperglycaemia, and AGE levels both in the serum and in a variety of tissues [4]. Of great interest was the further demonstration of a relationship between serum and tissue AGE levels and the severity of diabetic complications [5, 6]. It was thus hypothesized that AGE modification of proteins played a causal role in the development of diabetic complications. Preliminary results of studies utilizing aminoguanidine, an inhibitor of advanced glycation, to prevent diabetic complications seem to support this hypothesis [7].

The subsequent discovery that AGEs accumulate also in uraemic patients came as a great surprise. Observed serum AGE levels were indeed elevated more than 10-fold above those of diabetic patients and appeared unrelated to elevated glucose levels [8, 9]. This latter point was verified by the fact that, in contrast with diabetes, there is no correlation between serum levels of fructoselysine (mirroring elevated glucose levels) and AGEs [9].

Two questions then arose: what is the relevance of elevated AGE levels in uraemia, and which are the determinants of AGE accumulation in uraemic patients? The relevance of AGE accumulation in uraemic patients was promptly established by the demonstration of an AGE-modified β2m both in the serum of dialysed patients and in β2m amyloid deposits [10–12], a specific complication of chronic dialysis patients. Monoclonal and polyclonal anti-AGE antibodies specifically stained amyloid deposits in dialysis patients [10, 12]. It was further demonstrated that AGE-β2m was capable of attracting monocytes [13] and of stimulating monocyte-derived macrophages exhibiting AGE receptors to release a variety of cytokines [13–16]. Furthermore AGE-β2m stimulated osteoclast-induced bone resorption [17]. Preliminary evidence also suggests that AGEs play a critical role in the development of intimal hyperplasia in the vessels of uraemic subjects [our unpublished observation].

Elucidation of the factors responsible for AGE formation proved more difficult. Indeed AGEs are heterogeneous structures, some but not all of which have been identified. It thus became necessary to identify with greater precision the structures of AGEs accumulating in renal failure. Two such structures have been identified: pentosidine [18] and Nε-carboxymethyllysine (CML) [19] (Figure 1). Both are markedly elevated in uraemic patients irrespective of the presence of diabetes [9, and our unpublished observation]. Both have been specifically identified in β2m amyloid deposits [12, 20]. Finally, both are present in the AGE structures recognized in diabetic patients [4].

The genesis of AGEs including pentosidine and CML in non-diabetic uraemic patients remains to be clarified. As pentosidine is mainly linked to albumin in the serum [9], its accumulation cannot be attributed to a decreased removal by glomerular filtration.

Furthermore, as already stated, undetected fluctuations of blood glucose concentration cannot be invoked in the absence of any correlation between pentosidine and fructoselysine levels. Obviously, uraemic sera contain either unknown precursors and/or catalysts of the Maillard reaction.

Recently gathered evidence has led us to postulate that an increased oxidative stress, characteristic of chronic uraemia, stimulates the oxidation of a variety of precursor substances which are unable, under normal conditions, to augment AGE production. First, the formation of AGE products such as pentosidine and CML has been known for some years to be closely linked to ‘oxidation’ processes [21]. Figure 1 shows the Maillard reaction pathway leading to pentosidine and CML formation. CML was originally identified as a product formed by oxidative cleavage of a glucose-derived Amadori compound [19]. However, it was recently demonstrated that glyoxal, which is formed on autoxidation of glucose, is also an efficient precursor of CML [22–24]. Pentosidine was proposed to be formed spontaneously through a reaction of protein with ribose [18], but arabinose, another autoxidative...
product of glucose, is also a potential source of pentosidine [22, 23]. AGEs are thus products of the combined process of ‘glycation’ and ‘oxidation’. This contention is supported by evidence that, in vitro, the absence of oxygen in the incubation medium prevents pentosidine and CML formation [19, 25].

Second, it is of note that ascorbic acid is easily oxidized under oxidative stress and its oxidized form is an efficient precursor of both pentosidine and CML [25–27]. In vitro incubation of proteins with ascorbic acid under atmospheric oxygen, taken as oxidative stress, resulted in the rapid appearance of characteristic physicochemical properties of AGEs (brown colour, fluorescence, polymerization tendency) and the transformation into AGE-modified proteins recognized by a specific monoclonal antibody [35]. These results demonstrate that AGE production is accelerated under oxidative stress even in the absence of glucose, suggesting that, under oxidative stress, AGE production is also determined by the availability of precursors such as the oxidized form of ascorbic acid.

Third, chronic uraemia might be a state of increased oxidative stress, as suggested by increased lipid peroxidation assessed by malondialdehyde [28, 29], an increased ratio of oxidized glutathione to reduced glutathione [30, 31], an increased ratio of oxidized form of albumin to reduced form of albumin [32], and a decreased activity of glutathione-dependent enzymes such as glutathione S-transferase, glutathione reductase and glutathione peroxidase [31]. More recently, Witko-Sarsat et al. [33] demonstrated elevated levels of ‘advanced oxidation protein products (AOPP)’ (taken as highly oxidized proteins) in sera of uraemic patients. We recently found that, in patients undergoing chronic haemodialysis, the ratio of oxidized form of ascorbic acid to total ascorbate is increased. If this ratio is taken as a marker of the redox state, this finding indicates a significantly higher oxidative stress in haemodialysis patients than in normal subjects. Under these circumstances, there is a linear relationship between the plasma levels of oxidized ascorbic acid and pentosidine [35]. This relationship is absent in healthy individuals despite similar serum levels of oxidized ascorbic acid. It thus appears that, in uraemia, several precursors of pentosidine and possibly CML, normally not utilized, are suddenly incorporated into AGEs provided that the oxidative stress is increased.

The oxidative stress is a measure of the steady-state level of reactive oxygen or oxygen radicals in a biological system [24]. By definition it is highly variable from tissue to tissue, depending on local conditions, with overproduction of precursors to reactive oxygen radicals and/or decreased efficiency of inhibitory and scavenging systems. It may thus be predicted that oxidative stress with its attendant formation of AGEs will not be uniform in an individual patient either in location or in time. For instance, unlike serum pentosidine, haemoglobin-linked pentosidine is not elevated in uraemic blood [our unpublished observation], a finding compatible with the existence of a powerful antioxidant system within red blood cells. Pentosidine levels are also correlated with products of monocyte activation, a condition known to augment oxidative stress [34]. Thus, the hypothesis that an augmented oxidative stress accelerates AGE formation in uraemia opens new doors to the understanding of preferentially located AGE damage to various proteins in uraemic patients.

In conclusion, several lines of evidence support the hypothesis that oxidation conspires with glycation to generate noxious advanced glycation end products in renal failure (Figure 2). This hypothesis, if confirmed, might open the way to significant therapeutic approaches and developments in the prevention of complications associated with end-stage renal failure and chronic dialysis.

References


